

SHORT COMMUNICATION

Cytological Studies on *Stereosandra javanica*, a Myco-heterotrophic Orchid

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Cytological studies of the myco-heterotrophic orchid *Stereosandra javanica* Blume revealed a chromosome number of $2n = 116$ and 58II, reported here for the first time. The chromosome complement at resting stage was of the complex chromocenter type. Metaphase chromosomes showed a gradual decrease in chromosome length from 1.5 to 0.9 μm . The number, $2n = 116$, is the highest chromosome number in Japanese wild orchids hitherto recorded and differs from the chromosome numbers in the closely related genus *Epipogium*; $2n = 72 = 36\text{II}$ in *E. aphyllum* Sw., $2n = \text{ca. } 70 = 35\text{II}$ in *E. roseum* (D. Don) Lindl. and $2n = 78$ in *E. japonicum* Makino.

Key words: chromosome, Epipogiinae, karyotype, myco-heterotrophic, Orchidaceae, *Stereosandra javanica*

Although ornamental epiphytic orchids have been studied cytologically for a long time, such studies of myco-heterotrophic orchids are still rare due to the difficulty of collecting actively growing meristematic tissues. The local and rare occurrences, few individual plants, short above-ground growing season, and the difficulty in cultivating myco-heterotrophic orchids, makes them especially difficult to study experimentally. Thus, only 15 of the 37 myco-heterotrophic orchids in Japan have been studied cytologically.

Schlechter (1926) established the subtribe Epipogiinae ('Epipogoneae') in which he included two genera, *Stereosandra* and *Epipogon* (a synonym of *Epipogium*). Subsequently, Dressler (1993) recognized subtribe Epipogiinae, which included three genera; *Epipogium*, *Stereosandra* and the newly described *Silvorchis*. *Stereosandra*, a monotypic genus, differs from *Epipogium* by lacking spurs. The single species, *Stereosandra javanica* Blume, is distributed widely in tropical and subtropical Asia. In Japan, it is rare in *Castanopsis* forests in the Ryukyu Islands. In this paper, the result of a cytological analysis of *S. ja-*

vanica is reported and its karyotype is compared with the karyotypes of reported for *Epipogium*.

Materials and Methods

Two plants of *Stereosandra javanica* (Japanese name: Iriomote-muyoran) used in this study

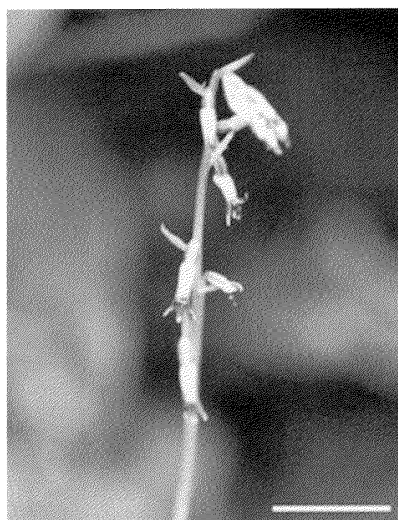


FIG. 1. Flowering *Stereosandra javanica* Blume in natural habitat in early June 2010. Bar = 20 mm.

were collected at an altitude of approximately 200 m on Ishigaki Island, Okinawa Prefecture, Japan, in early June, 2010 (Fig. 1). A voucher specimen (*M. Aoyama s.n.*) has been deposited in the herbarium of the Faculty of Science, University of Ryukyus (RYU).

For observations, young growing flower buds of both plants were collected from the upper parts of the inflorescences. The ovaries were cut into 1–2 mm pieces. The pieces were immersed in 2 mM 8-hydroxyquinoline at 12°C for 5 h. They were subsequently fixed in acetic alcohol (1:3) at 5°C for 24 h. The fixed materials were hydrolyzed in a 1:2 mixture of 45% acetic acid and 1N HCl at 60°C for 30 s. Immature placental tissues with

young ovules were separated from the inside of the ovaries and placed on glass slides, stained in 2% aceto-orcein, then prepared for observation using the usual squash method.

Classification of the chromosome types at resting and mitotic prophase stages follows Tanaka (1977). Terminology of chromosome morphology at mitotic metaphase on the basis of the position of the centromere follows Levan *et al.* (1964).

Results and Discussion

The young ovules showed both mitosis in the integument tissues and meiosis in the embryo sac mother cells. At resting stage, the chromosomes

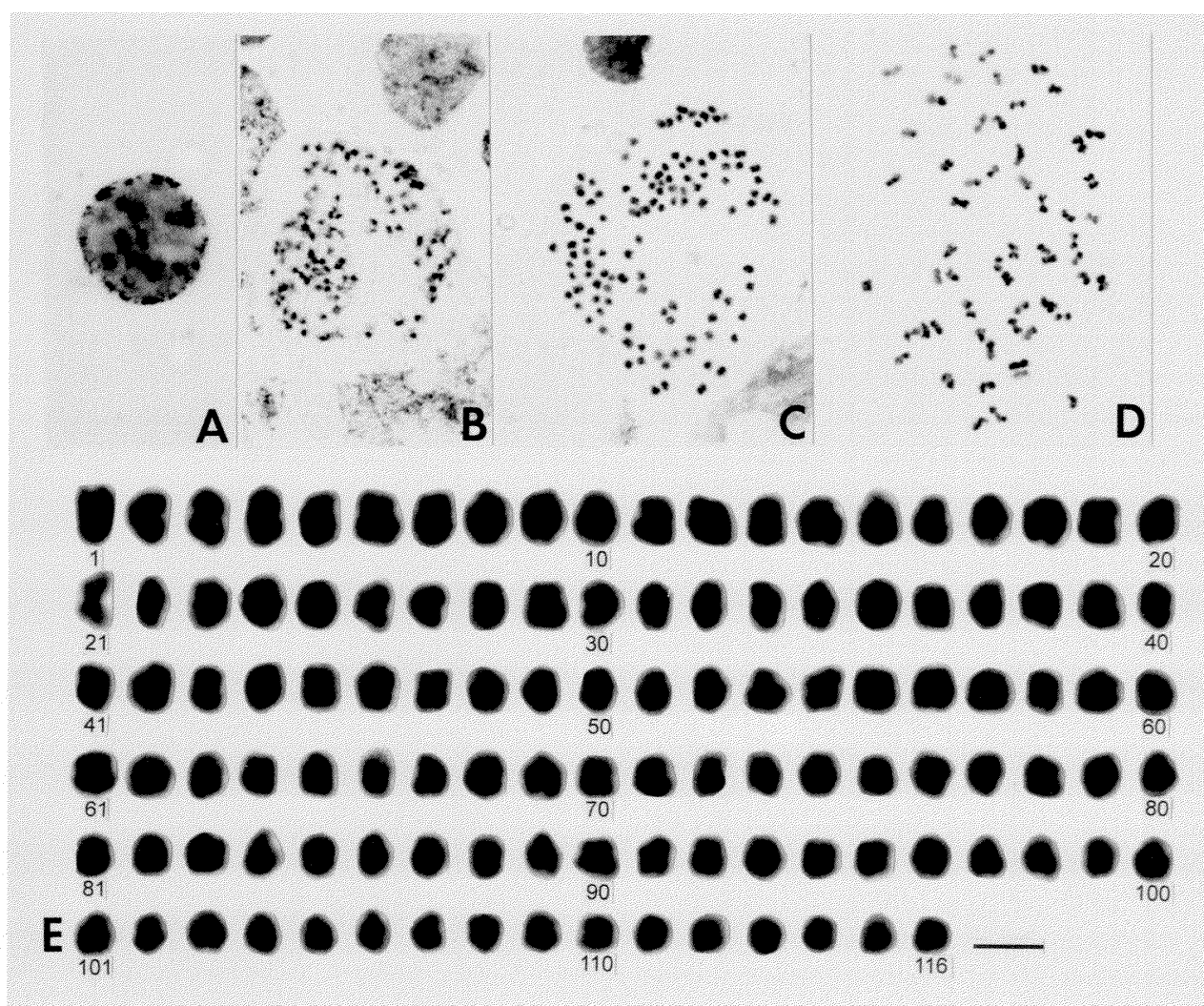


FIG. 2. Orcein-stained chromosomes of *Stereosandra javanica* Blume. A: Resting stage. B: Mitotic prophase. C: Mitotic metaphase, $2n = 116$. D: Meiotic metaphase I, $2n = 58\text{II}$. E: Chromosome alignment at mitotic metaphase by length. Bar indicates 10 μm for A–D and 2 μm for E.

TABLE 1. Comparison of chromosome characters in four species of subtribe Epipogiinae.

Species	Karyotype at resting stage	Karyotype at metaphase	Chromosome number	Chromosome length largest–smallest (μm)	Total length (μm)	Average length (μm)
<i>Stereosandra javanica</i>	complex chromocenter	gradual	$2n = 116$, 58II	1.5–0.9	129	1.1
<i>Epipogium aphyllum</i> *	complex chromocenter	bimodal	$2n = 72$, 36II $n = 36$	6.5–1.4	140	1.9
<i>E. roseum</i> *	complex chromocenter	gradual	$2n = \text{ca. } 70$, 35II $n = 35$	1.7–0.7	66	0.9
<i>E. japonicum</i> **	diffused type	gradual	$2n = 78$	2.0–1.2	119	1.5

From *Aoyama (2011) and **Aoyama (2008)

of *Stereosandra javanica* formed approximately 15 darkly stained chromatin blocks per nucleus (Fig. 2A). The chromosomes at resting stage were characterized as complex chromocenter type. The chromosomes at mitotic prophase formed early condensed segments that were usually located in the proximal region (Fig. 2B).

A chromosome number of $2n = 116$, the highest number in Japanese wild orchids so far reported, was uniformly counted in five cells of both plants at mitotic metaphase (Fig. 2C). Fifty eight bivalent chromosomes were counted in three embryo sac mother cells at meiotic metaphase I (Fig. 2D). These are the first reports of the chromosome number for the genus *Stereosandra*. The chromosomes at mitotic metaphase varied in length from 0.9 to 1.5 μm (Fig. 2E). The sum of the chromosome lengths was 129 μm . Among the 116 chromosomes, 35 were median centromeric, 16 were submedian centromeric, ten were subterminal centromeric. The position of the remaining 55 centromeres could not be determined. According to the karyotype definitions proposed by Tanaka (1977), *S. javanica* showed a homogeneous and gradual karyotype due to a gradual decrease in chromosome length.

Along with the karyotypes of three species of *Epipogium* recently reported (Aoyama 2008, 2011), these four species of orchids differed from each other in the following characteristics; karyotypes at resting stage, karyotypes at metaphase,

chromosome numbers, largest and smallest chromosome lengths, total chromosome lengths and average chromosome length (Table 1). The high chromosome numbers in subtribe Epipogiinae, however, are a common feature among the members of Orchidaceae. Aoyama (2011) suggested that the chromosome number $2n = 72$ in *E. aphyllum* Sw. might be derived from $2n = 78$ by chromosome fusion, and that *E. aphyllum* and *E. japonicum* Makino are karyomorphologically more closely related to each other than either is to *E. roseum* (D. Don) Lindl. The chromosome number of $2n = 116$ in *S. javanica* is clearly different from the number in *Epipogium*. The karyotypes at resting stage and metaphase of *S. javanica* are both the same as in *E. roseum*. The average chromosome length of 1.1 μm in *S. javanica* is closer in length to the 0.9 μm observed in *E. roseum* than to the lengths in *E. aphyllum* and *E. japonicum*. The chromosome number and total length in *S. javanica* is approximately twice the number and length in *E. roseum*. The findings suggest that *S. javanica* is derived from *E. roseum* by chromosome doubling and subsequent chromosome loss. Further information is needed to clarify the phylogeny of subtribe Epipogiinae.

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